JCM Journals.ASM.org

Next-Generation Sequencing of Prostate Tumors Provides Independent Evidence of Xenotropic Murine Leukemia Virus-Related Gammaretrovirus Contamination

In 2006, xenotropic murine leukemia virus (MLV)-related gammaretrovirus (XMRV) was isolated from prostate cancer tissue (9). However, subsequent studies have yielded conflicting and controversial results, with widespread detection of XMRV suggested to be the result of contamination with mouse DNA (3). While recent work has focused on PCR-based targeted detection of XMRV (10), the advent of next-generation sequencing (NGS) means that it is now possible to interrogate the entire genomes and transcriptomes of human samples for the unique genomic signatures of thousands of viruses (1, 5, 6).

With this in mind, we analyzed whole-genome (DNA-Seq) and transcriptome (RNA-Seq) data from 9 human prostate tumors (6 primary and 3 metastatic), 3 prostate tumor-derived murine xenografts, and 1 benign tissue sample from a pelvic lymph node. The xenograft tumors carried significant amounts of host mouse tissue, thereby acting as positive controls. We mapped all nonhuman RNA-Seq reads to a custom database of 3,932 viral genomes and 1,387 microbial genomes downloaded from the National Center for Biotechnology Information RefSeq database (July 2011) (7) and filtered for reads mapping specifically to MLVs, the family that includes XMRV. Murine genomes are hosts to hundreds of retroviruses (8); consistent with this, the xenograft tumor transcriptomes yielded thousands of MLV reads. Interestingly, 2 primary tumors (P3 and P5) also demonstrated significant enrichment of 5 MLVs, especially XMRV, in their transcriptomes, compared to the other patient tumors ($P = 1.28 \times 10^{-8}$ [t test comparing XMRV reads per million]) (Table 1).

To search for evidence of viral integration into the tumor genomes of P3 and P5, we mapped the nonhuman DNA-Seq reads to the same viral database. As expected, the xenograft samples showed enrichment of MLV DNA sequences. However, no DNA-Seq reads from patients P3 and P5 mapped to any of the MLVs (Table 1). Note that our DNA-Seq human genome coverage averaged >4×, while XMRV is expected to integrate on every chromosome within infected genomes (4).

Finally, we interrogated RNA-Seq data for 2 mouse-specific segments of the mitochondrial *cytB* gene (mouse mitochondrial DNA [mtDNA]; NC_005089.1). The xenograft samples were strongly positive, but tumors P3 and P5 also demonstrated enrichment of mouse mtDNA (Table 1). Indeed, the correlation coefficient across all human samples between the numbers of reads per million mapping to XMRV mtDNA and to mouse mtDNA was 0.982 (note that there was no sequence common to the XMRV mtDNA and mouse mtDNA).

To our knowledge, this is first analysis of XMRV in NGS data and provides no evidence for XMRV infection in human prostate tumors. The 2 human samples with enrichment of XMRV reads were also enriched with mouse mtDNA, suggesting a contaminant origin for the viral reads in the samples from those patients. Interestingly, the RNAs from these two samples were extracted at similar time points distinct from those of the other tumor samples and the DNA extractions. The recent emergence of large-scale sequencing projects such as the International Cancer Genome Consortium (2) means that it should soon be possible to mine

Published ahead of print 7 December 2011
Address correspondence to Colin C. Collins, ccollins@prostatecentre.com.

doi:10.1128/JCM.06170-11

TABLE 1 Analysis of RNA-Seq and DNA-Seq reads from human prostate tumors mapping to viral genomes[a]

Sample	RNA-Seq					DNA-Seq			
		No. of reads per million					No. of reads per million		
	No. of nonhuman reads (× million)	RefSeq viral genomes	MLVs	XMRVs	Mouse-specific sequences	No. of nonhuman reads (× million)	RefSeq viral genomes	MLVs	XMRVs
P1	26.5	17.86	4.15	0	0	16.2	21.77	0	0
P2	28.6	46.61	3.5	1.11	1.07				
P3	31.8	118.54	22.46	11.25	4.99	9.3	15.53	0	0
P4	22.9	8.77	4.71	0.04	0.73				
P5	23.5	111.43	21.19	10.97	6.33	36.3	2.56	0	0
P6	16.5	99.01	0.84	0	0.36				
M1[b]	25.8	40.03	4.78	0.87	0.58				
M2	29	22.91	4.33	1.47	1.23				
M3	15.7	22.58	0.63	0	0.38				
M4	15.5	18.04	3.01	0	0				
X1	29.9	2,187.91	327.27	169.45	1,251.12	53.3	30.82	10.07	4.11
X2	71.3	1,644.88	421.93	192.94	162.75	59.6	22.32	5.97	2.38
X3	21.3	2,990.69	443.02	220.13	65.92				

[a] "Nonhuman reads" refers to reads that could not be mapped to the human genome. P, primary prostate tumor; M, lymph node metastasis; X, human-derived xenograft tumor. Note that metastatic samples M1 to M4 were isolated from the same patients as tumor samples P1 to P4.
[b] Adjacent benign tissue was sampled.

large data sets in a manner similar to that described here and facilitate the detection of XMRV in cases of prostate cancer.

ACKNOWLEDGMENTS

We thank Canada's Michael Smith Genome Sciences Centre at the BC Cancer Agency for providing sequencing services using Illumina technology and Dong Lin and Yuzhuo Wang for providing xenograft tissue.

REFERENCES

1. **Cheval J, et al.** 2011. Evaluation of high-throughput sequencing for identifying known and unknown viruses in biological samples. J. Clin. Microbiol. **49**:3268–3275.
2. **Hudson TJ, et al.** 2010. International network of cancer genome projects. Nature **464**:993–998.
3. **Katzourakis A, Hue S, Kellam P, Towers GJ.** 2011. Phylogenetic analysis of murine leukemia virus sequences from longitudinally sampled chronic fatigue syndrome patients suggests PCR contamination rather than viral evolution. J. Virol. **85**:10909–10913.
4. **Kim S, et al.** 2008. Integration site preference of xenotropic murine leukemia virus-related virus, a new human retrovirus associated with prostate cancer. J. Virol. **82**:9964–9977.
5. **Loh J, et al.** 2009. Detection of novel sequences related to African swine fever virus in human serum and sewage. J. Virol. **83**:13019–13025.
6. **Palacios G, et al.** 2008. A new arenavirus in a cluster of fatal transplant-associated diseases. N. Engl. J. Med. **358**:991–998.
7. **Pruitt KD, Tatusova T, Klimke W, Maglott DR.** 2009. NCBI reference sequences: current status, policy and new initiatives. Nucleic Acids Res. **37**:D32–D36.
8. **Stocking C, Kozak CA.** 2008. Murine endogenous retroviruses. Cell. Mol. Life Sci. **65**:3383–3398.
9. **Urisman A, et al.** 2006. Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog. **2**:e25.
10. **Yang J, Battacharya P, Singhal R, Kandel ES.** 2011. Xenotropic murine leukemia virus-related virus (XMRV) in prostate cancer cells likely represents a laboratory artifact.Oncotarget **2**:358–362.

Fan Mo
Alexander W. Wyatt
Chunxiao Wu
Anna V. Lapuk
Vancouver Prostate Centre & Department of Urologic Sciences
University of British Columbia
Vancouver, BC, Canada

Marco A. Marra
Canada's Michael Smith Genome Sciences Centre
BC Cancer Agency
Vancouver, BC, Canada

Martin E. Gleave
Stanislav V. Volik
Colin C. Collins
Vancouver Prostate Centre & Department of Urologic Sciences
University of British Columbia
Vancouver, BC, Canada